Remarks

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Claims 1, 4, 5, and 53 have been amended; claims 3, 23-26, and 32-46 have been cancelled without prejudice, and new claim 54 has been added. Descriptive support for the amendments to claims 1 and 53 and new claim 54 is found, *inter alia*, in original claim 3, Figures 5 and 6, and paragraphs [0025], [0026], [0039], [0115] with Table 1, and [0123]. No new matter has been added by way of the above amendments.

The withdrawal of claim 11 is improper. Claim 11 recites a feature that is present *in combination with* the features of claim 1. Restriction as between a combination and sub-combination is improper and should be vacated.

Claims 1, 4-6, 9, 12, 13, 19-22, 53, and 54 are under examination (claim 54 should be examined with the elected subject matter), and claims 11, 14-18, and 48-52 stand withdrawn. No excess claim fees are due with this submission.

The rejection of claims 1, 3-6, 9, 12, 13, 19-22, and 53 under 35 U.S.C. § 112 (1st paragraph) for failing to comply with the written description requirement is respectfully traversed.

The United States Patent and Trademark Office ("PTO") asserts that the specification does not support the full scope of Factor VIII proteins comprising an A1 domain which has point mutations in or near a calcium binding site. The PTO further asserts that it would require undue experimentation for a person of skill in the art to ascertain which mutations near the calcium binding site would lead to higher specific activity.

Claim 1 has been amended to recite that the recombinant factor VIII comprises "an A1 domain that includes a substitution of a glutamic acid residue at the fourth position of a calcium binding site of the A1 domain."

Claim 53 has been amended to recite that the recombinant factor VIII comprises "an A1 domain having a calcium binding site according to one of SEQ ID NOS: 4-7 except that the calcium binding site has a substitution of the glutamic acid residue at the fourth position thereof." Each of SEQ ID NOS: 4-7 contains a glutamic acid residue at the fourth position.

Applicants submit that the application provides adequate written descriptive support for recombinant factor VIII molecules of the presently claimed invention, because the

structure and function of wild type factor VIII proteins was well known in the art, the A1 domain calcium binding sites are illustrated in the application as having a highly conserved structure and function (*see* Figures 5 and 6), and the glutamic acid residue at the fourth position of the calcium binding site (i.e., at position 113 of the human wild-type factor VIII) is conserved among the illustrated wild-type A1 domain calcium binding sites (*see* Figure 6).

As evidence that the structure and function of factor VIII proteins was well known in the art, the specification identifies wild-type factor VIII nucleic acid and amino acid sequences from human, rat, mouse, dog, chimp, and pig by their Genbank accessions. These Genbank accessions are incorporated by reference into the specification. The specification also references the availability of homology and alignment analyses for factor VIII proteins via the HAMSTeRS internet site (see paragraph [0033]). Attached as Exhibit 1 to applicants' prior submission, dated February 24, 2009, is the referenced HAMSTeRS alignment of human, porcine, murine, and canine factor VIII. That alignment demonstrates that the high degree of homology among these proteins was known in the art at the time of filing.

With respect to the human sequence, which for the above-noted reasons represents the genus of mammalian factor VIII, the sequences and domains (A1, A2, B, A3, C1, C2) are explicitly identified in paragraphs [0034] – [0038]. These domains are shared by all of the mammalian factor VIII proteins.

As evidence of the highly conserved structure and function of mammalian factor VIII, the specification also identifies a number of prior art, chimeric, recombinant factor VIII proteins that can be modified to include the modified calcium binding site of the present invention. These chimera are identified in paragraph [0045] of the present application, and described in U.S. Patent Nos. 5,859,204 and 6,770,744 to Lollar et al. as well as U.S. Patent Application Publ. No. 2003/0166536 to Lollar et al. Each of these references is incorporated by reference in its entirety. The chimera demonstrate that the structure and function of the human and porcine factor VIII sequences are so similar that a number of amino acid residues affecting antigenicity of the *porcine* sequence can be replaced with a corresponding residue from the corresponding human sequence without substantially altering the activity of the chimeric factor VIII with respect to human factor IX and human factor X.

Moreover, the specification at Figure 5 demonstrates that the factor VIII A1 domain calcium binding site bears striking homology to the previously known Factor V calcium binding site (*see also* paragraphs [0115] and [0123]). The factor VIII site shares all of the acidic

(Glu and Asp) residues of the factor V site. Thus, structure and function is, not surprisingly, conserved among calcium binding proteins. Furthermore, conservation of the structure and function of A1 domain calcium binding sites is identified in paragraph [0038] and illustrated in Figure 6, which shows an alignment of corresponding regions of human, murine, porcine, and canine factor VIII (SEQ ID NOS: 4-7). Each of these sequences is characterized by a structure that is conserved with the structure of the known Factor V calcium binding site (all acidic Glu and Asp residues of the Factor V site appear in the correct position in SEQ ID NOS: 4-7). Thus, the specification demonstrates that the structure and function of wildtype A1 domain calcium binding sites is conserved among four different mammalian factor VIII proteins.

Examples 10 and 11 demonstrate the effect of modifying the acidic residues at the first (E110), fourth (E113), sixth (D115), seventh (D116), thirteenth (E122), fifteenth (E124), sixteenth (D125), and seventeenth (D126) positions of the A1 domain calcium binding site. Example 11 identifies the first (E110), seventh (D116), thirteenth (E122), sixteenth (D125), and seventeenth (D126) residues as forming a calcium-coordination site in the A1 domain. These residues are shared by all four of the wild-type mammalian factor VIII listed in Figure 6 and the factor V calcium binding site of Figure 5.

Moreover, all four of these wild-type mammalian factor VIII possess calcium binding sites that share a glutamic acid residue at the fourth position of the site (E113). It is the substitution of this glutamic acid residue that is demonstrated to result in the higher specific activity (relative to wild type), as measured in a one-stage clotting assay. Seven different substitutions are shown to achieve similar to modestly or significantly improved specific activity (*see* Figure 7 and Example 13).

Because persons of skill in the art were well aware of the conserved structure and function of wild type mammalian factor VIII proteins prior to the present invention, and the present application demonstrates that (i) the A1 domain calcium binding sites have a conserved structure and function, (ii) the glutamic acid residue at the fourth position of the calcium binding site is conserved among wild-type factor VIII proteins, and (iii) *seven* different glutamic acid substitutions are demonstrated to possess similar to modestly or significantly improved specific activity, persons of skill in the art would have appreciated that applicants were in possession of the claimed invention.

Despite this showing, the PTO asserts at page 4 of the outstanding office action that the application provides "minimal guidance as to which substitutions and which amino acids

are critical for the wild-type protein to have higher specific activity." Applicants disagree for the reasons noted above. More particularly, the present application identifies which glutamic acid residue to substitute and the array of different substitutions that can achieve the desired increase in specific activity. Given the results presented in the application, persons of skill in the art would have fully appreciated how to modify other wild type or recombinant/mutant factor VIII proteins to arrive at a recombinant factor VIII having increased specific activity.

The PTO's citation to *Fiers* and *Amgen* at page 4 of the office action is inapposite. It is a gross understatement to suggest that applicants have provided only "a mere statement" of the invention and "a potential method of isolating or making it." This verbiage quoted from these cases is entirely irrelevant to facts presented. Applicants *have made and tested* a number of specific recombinant factor VIII proteins, and this supports the invention as claimed. This is more than sufficient to demonstrate possession of the invention.

For these reasons, the rejection of claims 1, 3-6, 9, 12, 13, 19-22, and 53 for lack of written description should be withdrawn.

The rejection of claims 1, 3-6, 9, 12, 13, 19-22, and 53 under 35 U.S.C. § 112 (1st paragraph) for lack of enablement is respectfully traversed.

The PTO asserts at pages 4-6 of the office action that the specification is only enabling for position 113 mutants. Applicants have amended claims 1 and 53 to specify the position of the wild-type glutamic acid residue that bears the substitution.

However, the PTO asserts at page 5 of the office action that undue burden would be required to identify any other modifications to the factor VIII molecule in addition to the substitution in the A1 domain calcium binding site. Applicants disagree, because it is well known to persons of skill in the art that a number of modifications can be made which do not alter factor VIII activity, but instead alter another property of the factor VIII molecule (e.g., antigenicity, circulating half-life, protein secretion, affinity for factor IXa and/or factor X, altered factor VIII-inactivation cleavage sites, stability of the activated factor VIII form, immunogenicity, and shelf-life). A number of these known modifications are explicitly described in the specification at paragraphs [0041]-[0054], and the combination of any one or more of these known modifications with the recited calcium binding site substitution is *explicitly* contemplated *and demonstrated*. Specifically, the examples were achieved using a B-domainless factor VIII, which is the variant described in paragraph [0042]. Other variants include recombinant human/porcine factor VIII that possess modified antigenicity (*see* paragraph [0045]

listing 37 substitutions that achieve reduced antigenicity); recombinant factor VIII having enhanced stability of the activated form by virtue of A2-A3 domains fused via Cys664-Cys-1826 (*see* paragraph [0046]); recombinant factor VIII having altered factor VIII-inactivation cleavage sites at either Arg336 or Arg562 (*see* paragraph [0047]); recombinant factor VIII having an F309A/S point mutation, which enhances secretion of the recombinant factor VIII (*see* paragraph [0049]); recombinant factor VIII having reduced antigenicity following glycosylation at asparagines residues (*see* paragraph [0051]); recombinant factor VIII having a modification that results in procoagulant activity, including the deletion of the von Willebrand factor binding site, a mutation at R740, or added amino acid sequence spacer between the A2- and A3-domains, where the amino acid spacer is of a sufficient length so that upon activation, the procoagulant-active factor VIII protein becomes a heterodimer (*see* paragraph [0052]).

These explicit combinations are supported by the specification and certainly would not require undue experimentation, because these additional modifications were all known in the prior art to produce specific results and persons of skill in the art would have been fully able to make such a combination. The examples demonstrate as much using the E113 substitution in a B-domainless factor VIII. Thus, undue experimentation would not be involved in making and using other factor VIII mutants that include a modified A1 domain calcium binding site substation as claimed.

Because the factor VIII A1 domain calcium binding sites are demonstrated to share substantial homology (and, thus, are both structurally and functionally conserved), seven different embodiments are described and tested in a B-domainless variant, and other modifications to alter additional properties of factor VIII were known in the art, the enablement rejection of claims 1, 3-6, 9, 12, 13, 19-22, and 53 should be withdrawn.

The rejection of claims 1, 6, 9, 12, 13, and 19-22 under 35 U.S.C. § 112 (2nd paragraph) for indefiniteness is respectfully traversed in view of the above amendments. As explained above, the present invention is not limited to human factor VIII, but instead is applicable to any factor VIII molecule that possesses an A1 calcium binding domain having the glutamic acid residue at the fourth position of the domain.

The PTO asserts at page 7 of the office action that claim 1 is indefinite because the source and/or species from which the sequence is taken is not recited. PTO further asserts that it would be difficult to point out the position of mutations and calcium binding sites without referring to a specific sequence for Factor VIII. Applicants disagree insofar as the position of

calcium binding site in the A1 domain of Factor VIII proteins can be easily identified by a person of skill in the art using well known sequence comparisons. For example, Figure 5 illustrates an alignment between the factor V calcium binding domain and the human factor VIII calcium binding domain, and Figure 6 is an alignment of human, porcine, murine, and canine factor VIII. Figure 6 demonstrates the high conservation among mammalian Factor VIII calcium binding domains; and Figures 5 and 6 together demonstrate the absolute conservation of Glu and Asp residues of factor V (SEQ ID NO: 3) in all of the factor VIII A1 domain calcium binding sites. Consequently, a person of skill in the art would be fully apprised of the structure and function of a factor VIII A1 domain calcium binding site, as well as where the glutamic acid residue at the fourth position of this site resides.

For these reasons, the rejection of claims 1, 6, 9, 12, 13, and 19-22 for indefiniteness is improper and should be withdrawn.

New claim 54 recites that the recombinant factor VIII comprises "an A1 domain…[that] comprises a calcium binding site that is homologous to and shares Glu and Asp residues with SEQ ID NO: 3, and which calcium binding site further comprises a substitution of a wild-type Glu residue at the fourth position of the calcium binding site, wherein the substitution results in a recombinant factor VIII having a specific activity, as measured in a one-stage clotting assay, that is higher than that of a wild-type factor VIII." Applicants submit that claim 54 overcomes the several asserted bases of rejection for substantially the same reasons noted above.

This submission is accompanied by a petition for extension of time. All fees associated therewith should be charged to deposit account 14-1138. Any overpayment or underpayment should be applied to this same account.

In view of all of the foregoing, applicant submits that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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